In vivo functionality of autologous tissue engineered constructs based on SPCL scaffolds cultured with goat marrow cells

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Statement of Purpose:
Bone marrow stromal cells, seeded and *in vitro* cultured onto an appropriate scaffold material, have a great potential for the regeneration of bone tissue defects.
The aim of this study was to assess the *in vivo* osteogenic ability of cell-scaffold constructs based on goat marrow stromal cells (GMCs) and SPCL (a blend of corn starch with polycaprolactone) fiber mesh scaffolds at different stages of development. This type of constructs has demonstrated a very good *in vitro* functionality in several previous studies by our group[1,2]. For this purpose, cell-scaffold constructs, *in vitro* cultured for different time periods, were implanted in non-critical size femoral defects of adult goats, using an autologous approach.

Methods:
Scaffold material - Fiber-mesh based on SPCL, (starch/ polycaprolactone 30/70% wt) scaffolds with a porosity of ~75%, were cut into discs (ϕ6mm, *h*=2mm), sterilized in ethylene oxide and used as scaffold materials.
GMCs harvest and culture - GMCs were harvested from iliac crests of adult goats using a bone marrow aspiration needle and were cultured in basal medium: DMEM (Dulbecco's Modified Eagle's Medium) supplemented with goat serum (10%) isolated from goat peripheral blood.
In vitro culture of cell-scaffold constructs - Cells were cryopreserved, expanded and sub-cultured twice (P2) before seeded onto SPCL scaffolds (5E+05cells/scaffold). After *in vitro* seeding, cells were cultured in the presence of osteogenic supplements (10^{-8}M dexamethasone, 50µg/ml ascorbic acid, 10mM β-glycerophosphate) for 1 and 7 days prior to implantation. An *in vitro* control of the experiment was considered, in which cell proliferation, assessed by DNA quantification and alkaline phosphatase activity (ALP) levels were measured.
Implantation surgery - Non-critical size defects (ϕ 6mm and 3mm depth) were drilled in both femurs of 4 adult goats. Drill defects alone served as controls as well as defects filled with scaffolds without cells. After implantation, intravital fluorescence markers, namely xylenol orange (90mg/kg), calcein green (10mg/kg) and tetracycline (25mg/kg) were injected subcutaneously (after 2, 4 and 6 weeks, respectively) for bone formation and mineralization monitoring.
Harvesting samples after implantation - After 6 weeks of implantation, animal euthanasia was performed using an overdose of intravenous pentobarbital sodium 20%. The femurs were then removed and cut into single defect transversal sections. Sections were fixed in a formol solution, embedded in glycol methacrylate, cut into 30µm slides and observed at the fluorescence microscope. Additional sections were also stained with *Levai Laczko* to observe new bone formation.

Results/Discussion:
In general, it was not observed any significant inflammatory response to the implanted constructs or infection. Preliminary results indicate that the neoformation of bone occurred in all femoral defects. Most of femur drills (controls and with cell-SPCL constructs) were not easily detected 6 weeks after implantation due to an excellent regeneration process and an exuberant periosteal reaction in some cases. In order to expose the drills and access the inner region, bone was longitudinally cut (Figure 1).

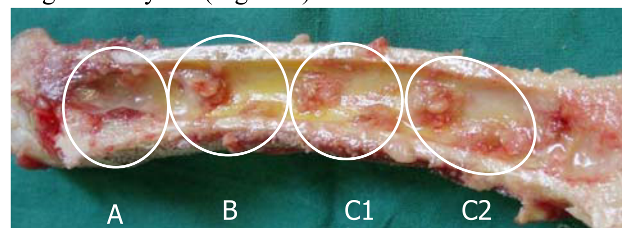

Figure 1. Image of the inner section of a femur 6 weeks after implantation; A) Control 1 – empty drill defects, B) Control 2 – defects filled with SPCL (no cells), C) defects filled with cell-SPCL constructs after C1) 1 day of culture and C2) 7 days of culture in osteogenic medium.

However, the observation of the femoral defects also suggests that the defects filled with cell-SPCL constructs seemed to have higher bone growth than empty drill defects or defects filled with SPCL materials alone. Furthermore, the same observations suggest some differences in bone formation depending on the *in vitro* culturing time of the implanted constructs, although this was not clearly observed in all samples retrieved.

Conclusions:
Neoformation of bone occurred in all femoral defects. Nevertheless, it seems that bone growth is definitely enhanced by the presence of cell-scaffold constructs. *In vitro* culturing time, i.e., the differentiation stage of GMCs also seems to play an important role in bone growth onto these defects.
Future studies should address bone formation in more complex models, namely bone critical size defects.

References:
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